Influence of the Lysine to Protein Ratio in Practical Diets on the Efficiency of Nitrogen Use in Growing Pigs[1]

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ABSTRACT : Twelve gilts were used to investigate the effect of lysine to protein ratio (5.2 g lysine/100 g CP vs. 6.7 g lysine/100 g CP) in practical diets on nitrogen retention and the efficiency of utilization in growing pigs. Treatments involved 2 levels of dietary lysine (5.2 or 6.7 g/100 g CP) and 3 levels of dietary crude protein (11, 14 and 17% in diet). Nitrogen retention was greatest when pigs were fed the control diet containing 17% protein. Nitrogen retention progressively increased as dietary protein increased ($p < 0.01$), but it was not affected by lysine concentration (g/100 g CP). Apparent biological value (ABV, nitrogen retained/apparently digestible nitrogen) was estimated to be ~50% at the maximum nitrogen retention. ABV was not affected by lysine concentration, but declined ($p < 0.05$) as the dietary protein level increased. The efficiency of intake N used for maximum nitrogen retention was approximately 44%. One gram of lysine supported approximately 9 to 10 g apparent protein accretion (nitrogen retention × 6.25/lysine intake) in pigs fed control diets. The efficiency of lysine utilization for protein accretion was lower in pigs fed high-lysine diets (6.7 g lysine/100 g CP) so that 1 g of lysine accounted for 7 to 8 g of protein accretion in these pigs ($p < 0.01$). The lysine required to support maximum nitrogen retention in pigs fed high-lysine diets was higher than that in pigs fed control diets, which suggests that lysine was over-fortified relative to crude protein, since practical diets can not be formulated without excess of some amino acids. In summary the concentration of 5.2 g total lysine/100 g CP in diet is more appropriate for corn-soybean diets than the commonly suggested the content of 6.7 g total lysine/100 g CP.

(**Key Words**: Lysine, Nitrogen Retention, Nitrogen Utilization, Growing Pig)

INTRODUCTION

For growing pigs to utilize dietary amino acids efficiently for protein deposition, amino acids should be adequate, but also well-balanced relative to need. The ARC (1981) proposed an ideal amino acid pattern for dietary protein, in which all indispensable amino acids were supposed to be in perfect balance and therefore, in the exact amounts needed to support maximum protein accretion in growing pigs. They established the proper proportions among indispensable amino acids and the proportions of lysine to total protein. This pattern was an approximation, however, since it was based on the composition of pig tissue, with adjustments for differences observed between experiments. Various patterns of indispensable amino acids for growing pigs have been suggested by others (Moughan and Smith, 1984; Yen et al., 1986a,b; NRC 1988; Wang and Fuller, 1989; Chung and Baker, 1992). The intent of an ideal protein is to estimate the requirements of all other indispensable amino acids after determining the requirement of lysine. The ratio of indispensable to dispensable amino acids at a given dietary protein level is also important and determined by the lysine content in an ideal dietary protein.

The optimum content of lysine in an ideal protein was estimated to be in the range of 6.5 to 7.0 g per 100 g CP, which is equivalent to the ratio of about 45:55 of indispensable to dispensable amino acids (ARC, 1981; Wang and Fuller, 1989). The ratio of lysine to crude protein ranges from approximately 4.5 to 5.5 g/100 g CP when corn and soybean meal, supplemented with small amounts of lysine, are used. Large amounts of synthetic lysine and other indispensable amino acids (e.g. threonine, tryptophan, methionine, isoleucine etc.) would have to be added to practical diet to match the amino acid pattern of

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an ideal protein having 6.5 to 7.0 g lysine/100 g CP (ARC, 1981; Wang and Fuller, 1989). For this reason, formulating to 6.7 g lysine/100 g CP in practical diets may result in lower efficiency of use than 5.5 g lysine. This study was conducted to investigate the effect of lysine to crude protein ratio (5.2 g lysine/100 g CP vs. 6.7 g lysine/100 g CP) in a practical diets for nitrogen retention and efficiency of lysine use in growing pigs.

MATERIALS AND METHODS

Animals and housing

Twelve crossbred female pigs (Large White × Landrace × 'multiple-breeds' hybrid; PIC USA, Franklin, KY, USA) were obtained from the Cornell University Swine herd. Pigs were housed in an environmentally controlled room (20 ± 1 ℃ and 16:8 h light : dark cycle) and individually fed in metabolic cages during the experimental period. Foley catheters (14 Fr., 15cc; Rüsch Manufacturing Ltd, Germany) were introducted into the bladders. Average body weight was 59 ± 2 kg (mean ± SEM) on d1 when the digestion study began and 69 ± 3 kg (mean ± SEM) during the sampling period. The animal use protocol was approved by the Cornell University Animal Care and Use Committee.

Diets and treatments

The experimental diets are shown in table 1. The profile of indispensable amino acids approximated the ideal protein amino acid pattern of Wang and Fuller (1990) as follows : methionine+cystine≥61, threonine ≥65, tryptophan≥19, isoleucine≥65, valine≥75, leucine≥100, phenylalanine+tyrosine≥100 and histidine≥32, relative to lysine set equal to 100. The diets were in excess of some amino acids (i.e. histidine, leucine, phenylalanine+tyrosine) because it is difficult to perfectly match the ideal pattern with a combination of corn, soybean and synthetic amino acids without expensive use of synthetics. Spilled feed was collected, combined and analyzed to adjust N intake. Pigs were fed 6 times daily (07:00, 10:00, 13:00, 16:00, 19:00 and 22:00 h) with semi-automatic feeders at a total of 80 g/kg $BW^{0.75}$. Daily energy intake was 280 kcal DE/kg $BW^{0.75}$, which is equivalent to 2.6 times the maintenance energy requirement (NRC, 1988). The pigs had free access to water.

Twelve female pigs were divided into six pairs with similar body weight. Each pair of pigs received all six dietary treatments during the 3 week period (3 treatments/pig). Each pig received one treatment per week. Treatments included 2 levels of dietary lysine (5.2 or 6.7 g/100 g CP) and 3 levels of dietary protein (11, 14 and 17% in diet). The order in which pigs received treatments was decided using a modified 6×6 Latin Square Table (Lentner and Bishop, 1993).

Urine and feces collection

The experimental diets were first given 7 days after pigs were placed in metabolism cages (Day 1 of treatment). Catheters were introduced on d 5. Feces were collected on d 8 of treatment through d 13 (n=2 per treatment). Urine was collected from d 9 to d 13 for the first diet, from d 16 to d 20 for the second diet, and from d 23 to d 27 for the third diet. A 5-d acclimation period for bladder catheters was used prior to the first urine collection. A 3-d transition period was used to minimize carry-over effects between treatments. Total feces were collected every two hours from 07:00 to 23:00 h daily during the collection period and stored at −20 ℃. Later, a 5-d collection of feces was thawed, combined, weighed and homogenized. Subsamples were stored at −20 ℃ for future analysis. Urine was collected daily in 20-liter closed plastic jugs containing 400 ml of 10% HCl (v/v) to prevent loss of ammonia and microbial growth (pH < 3). All aliquots (10%) were stored at −20 ℃. Urine aliquots from 4 d were combined and stored at −20 ℃ for future analysis.

Chemical analysis

Nitrogen content of diets, spilled feed, urine and feces were assayed by Kjeldahl analysis in quadruplicate (AOAC, 1980). Wet feces were used to prevent loss of volatile N with drying as described by Van Soest and Robertson (1985). The energy content of diets and feces were obtained using an oxygen bomb calorimeter (Parr Adiabatic Calorimeter, Parr Instrument Co., Moline, IL.). Dry matter contents of diet and spilled feed were determined by drying samples at 65 ℃ until a constant weight was attained. Amino acids of corn and soybean meal were assayed using high performance liquid chromatography (Beckman Model 334 Gradient Liquid Chromatography System, Beckman Instrument Inc., Altex Division, San Ramen, CA). Acid hydrolysis was done on fat extracted samples using 6N HCl at 110 ℃ for 21 hours. Tryptophan was determined with alkaline (60% barium hydroxide) hydrolysate. For determining methionine and cystine, samples were first treated with performic acid before acid hydrolysis. Norleucine was used as internal standard. Amino acid contents of experimental diets were calculated using the amino acid contents of corn and soybean meal (table 1).

Table 1. Composition of experimental diets[a]

Item	Concentration					
	Control diets[b]			High-lysine diets[b]		
CP (%)	11	14	17	11	14	17
Ingredient (%)						
Corn	85.72	78.54	71.36	85.72	78.54	71.36
Soybean meal (48.5%)	8.38	15.57	22.76	8.38	15.57	22.76
Corn oil	1.49	1.49	1.48	1.49	1.49	1.48
Dicalcium phosphate	1.49	1.24	0.88	1.49	1.24	0.88
Limestone	0.81	0.90	0.99	0.81	0.90	0.99
Antibiotics[c]	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin-trace mineral premix[d]	0.25	0.25	0.25	0.25	0.25	0.25
L-Lysine-HCl	0.11	0.06	0.01	0.33	0.34	0.35
DL-Methionine	0.10	0.13	0.16	0.21	0.27	0.33
L-Threonine	0.02	0.01	0.01	0.13	0.16	0.19
L-Tryptophan	0.01	0.01	–	0.06	0.05	0.05
L-Isoleucine	–	–	–	0.11	0.13	0.16
L-Valine	–	–	–	0.13	0.16	0.20
L-Glycine	0.23	0.29	0.36	–	–	–
L-Glutamic acid	0.48	0.61	0.74	–	–	–
Nutrient						
DE (Mcal/kg)[e]	3.40	3.50	3.58	3.43	3.51	3.50
Crude protein (%)[f]	11.3	14.3	17.5	11.3	14.4	17.5
Calcium (%)[g]	0.80	0.80	0.80	0.80	0.80	0.80
Phosphorus (%)[g]	0.60	0.60	0.60	0.60	0.60	0.60
Amino acids (%)[h]						
Lysine	0.57	0.72	0.87	0.74	0.94	1.14
Met. + Cys.	0.35	0.44	0.52	0.45	0.57	0.69
Threonine	0.37	0.46	0.55	0.48	0.60	0.73
Tryptophan	0.11	0.14	0.18	0.14	0.19	0.23
Isoleucine	0.37	0.50	0.62	0.47	0.63	0.78
Valine	0.46	0.58	0.70	0.58	0.74	0.89
Leucine	0.96	1.12	1.29	0.96	1.12	1.29
Phe. + Tyr.	0.76	0.98	1.20	0.76	0.98	1.20
Histidine	0.26	0.32	0.39	0.26	0.32	0.39
IAA : CP (g/100g)[i]	37	37	36	43	42	42
IBIAA : CP (g/100g)[j]	31	31	31	40	40	40

[a] Presented on an as-fed basis.
[b] Designed lysine content in dietary protein was 5.2% for control diets and 6.7% for high-lysine diets.
[c] Provided the following antibiotics per kilogram of complete diet: Chlortetracycline-HCl, 110 mg; Sulfamethazine, 110 mg; Penicillin, 55 mg.
[d] Provided the follwoing nutrients per kilogram of complete diet; vitamin A, 5,510 IU; vitamin D, 1,320 IU; vitamin E, 20 IU; vitamin K, 2.2 mg; pantothenic acid, 17.6 mg; riboflavin, 4.4 mg; niacin, 35.2 mg; choline 95.6 mg; vitamin B_{12}, 25.5 μg; Mg, 270 mg; Zn, 80 mg; Fe, 80 mg; Mn, 40 mg; Cu, 10 mg; I, 1 mg; Se, 0.3 mg.
[e] Measured by the digestion study.
[f] Analytical value from Kjeldahl assay (AOAC, 1980).
[g] Calculated values (NRC, 1988).
[h] Calculated from analytical values of corn and soybean meal respectively as follows : (%), Lysine 0.254, 2.99; Methionine + cystine 0.182, 0.941; Tryptophan 0.042, 0.645; Threonine 0.249, 1.59; Isoleucine 0.236, 2.00; Valine 0.335, 2.05; Leucine 0.215, 3.71; Phenylalanine + tyrosine 0.552, 3.05; Histidine 0.198, 1.06.
[i] Calculated as 100 × (sum of indispensable amino acids/CP). IAA, indispensable amino acids.
[j] Calculated as 100 × (total ideally balanced indispensable amino acids/CP). IBIAA, the ideally balance indispensable amino acids in which the proportion of indispensable amino acids in excesses relative to an ideal protein pattern is not included. For example, leucine in the control diet containing 11% CP is in excess (168% of lysine) as compared to leucine in an ideal protein pattern (100% of lysine). Therefore, a proportion of leucine (68% of lysine) in excess is not included in IBIAA for the control diet containing 11% CP.

Statistical analysis

Six samples of urine and two samples of feces per treatment were obtained. Since four samples of urine output were missing from a pair of pigs after a feces collection was completed, urine samples obtained from this pair was excluded from analysis. Hence, a total of thirty observations (n=5 per treatment) were analyzed by two-way analysis of variance with a 2 × 3 factorial arrangement of treatments (lysine, protein and the interaction) for urine N data (Minitab, 1991; Lentner and Bishop, 1993). Average values for fecal N (n=2 per treatment) were used to determine the amounts of fecal N output. Apparently digestible (or absorbed) N was calculated as N in feed minus N in feces. Data are presented as treatment means.

RESULTS

The effect of lysine content on daily N flow is illustrated in table 2. Nitrogen retention was maximized at 0.98 g/kg$^{0.75}$per d when pigs were fed the control diet containing 17% protein. This is equivalent to 23.4 g of N retention per d for pigs weighing 69 kg (table 3). Nitrogen retention progressively increased as the level of protein increased (figure 1, $p < 0.01$), but was not affected by lysine concentration (g/100 g CP).

Apparent biological value (ABV, nitrogen retained/fecal digestible nitrogen) was estimated to be ~50% at maximum N retention. ABV was not affected by the lysine concentration, but declined ($p < 0.05$) as the dietary protein level increased (table 2). The efficiency of ingested N use for maximum N retention was approximately 44%. The efficiency of N utilization for N retention was not different among pigs fed diets containing different levels of protein or lysine concentration ($p > 0.10$).

Table 2. Effects of lysine content in ideal protein on daily nitrogen flow of growing pigs[a]

Criterion	Control diets			High-lysine diets			SEM	Statistical
CP (%)	11	14	17	11	14	17	(n = 5)[b]	significance[c]
N intake (g/kg BW$^{0.75}$)	1.44	1.82	2.22	1.43	1.85	2.26	0.01	$p < 0.01$
Fecal N (g/kg BW$^{0.75}$)	0.27	0.21	0.25	0.26	0.22	0.23	ND[d]	
Urinary N (g/kg BW$^{0.75}$)	0.48	0.74	0.98	0.50	0.73	1.08	0.07	$p < 0.01$
N retention (g/kg BW$^{0.75}$)	0.69	0.87	0.98	0.67	0.90	0.94	0.07	$p < 0.01$
ABV (%)[e]	59.2	54.1	50.0	57.5	55.2	46.5	3.7	$p < 0.01$
Gross N efficiency (%)[f]	48.0	47.7	44.3	46.9	48.9	41.7	3.3	$p < 0.10$

[a] Data are presented as treatment means. [b] Standard error of mean.
[c] Significant among protein levels. No significant lysine level or the interaction (protein × lysine) effects were observed.
[d] Not determined. Two pigs per diet were used for the digestion study.
[e] Apparent biological value = 100 × (N retention/digestible nitrogen).
[f] Gross Efficiency of N use for N retention = 100 × (N retention/N intake).

Table 3. Effect of lysine content in ideal protein on efficiency of lysine utilization for nitrogen retention or protein accretion in growing pigs[a]

Criterion	Control diets			High-lysine diets			SEM	Statistical
CP (%)	11	14	17	11	14	17	(n = 5)[b]	significance[c]
Body weight (kg)	67.0	69.1	68.7	67.4	69.2	68.9	2.6	
Lysine intake (LI; g/d)	10.3	13.3	16.1	13.5	17.6	21.3	0.5	P**, L**
N retention (NR; g/d)	16.1	20.1	23.4	15.7	21.7	22.4	1.7	P*
Lysine efficiency for N retention (NR/LI)	1.56	1.57	1.45	1.17	1.23	1.06	0.10	L**
Apparent Protein accretion (NR × 6.25)	101	126	146	98	135	140	11	P*
6.25 × NR/LI[d]	9.8	9.8	9.1	7.3	7.7	6.6	0.6	L*

[a] Data are presented as treatment means. [b] Standard error of mean.
[c] P, protein; L, lysine; ** $p<0.01$; * $p<0.05$. [d] Apparent protein accretion per unit of total lysine intake.

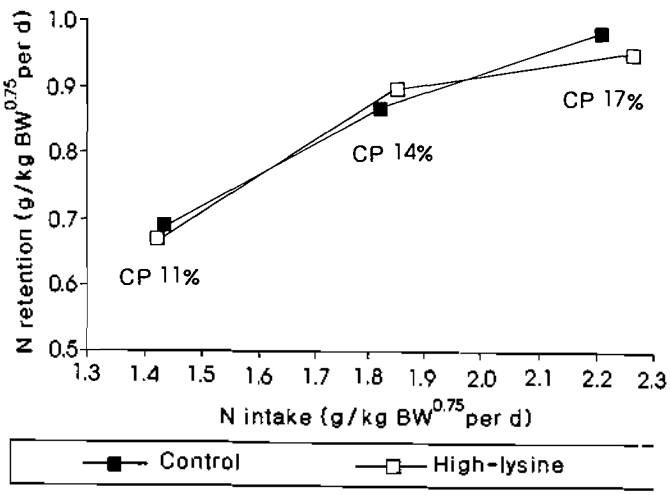

Figure 1. Effect of lysine content of diet on nitrogen retention in growing pigs weighing 69 kg. Symbols present treatment means (SEM = 0.07, n = 5). Lysine contents of control and high-lysine diets were 5.2 and 6.7 g per 100 g CP in the diets. Means for nitrogen retention were significantly affected by protein level ($p < 0.01$), but not by lysine content (g/100 g CP, $p = 0.89$) or the interaction ($p = 0.84$).

Nitrogen loss in the urine increased as N intake or the level of protein increased ($p < 0.01$). The efficiency of lysine utilization for N retention (nitrogen retention/lysine intake) ranged from 1.5 to 1.6 for pigs fed control diets containing 5.2 g lysine/100 g CP. One gram of lysine supported approximately 9 to 10 g protein accretion (nitrogen retention × 6.25/lysine intake, table 3) in pigs fed control diets. The efficiency of lysine utilization for protein accretion was lower in pigs fed high-lysine diets (6.7 g lysine/100 g CP) so that 1 g of lysine accounted for 7 to 8 g of protein accretion in these pigs ($p < 0.01$, table 3 and figure 2). The lysine required to support maximum N retention in pigs fed high-lysine diets was higher than that in pigs fed control diets (figure 3).

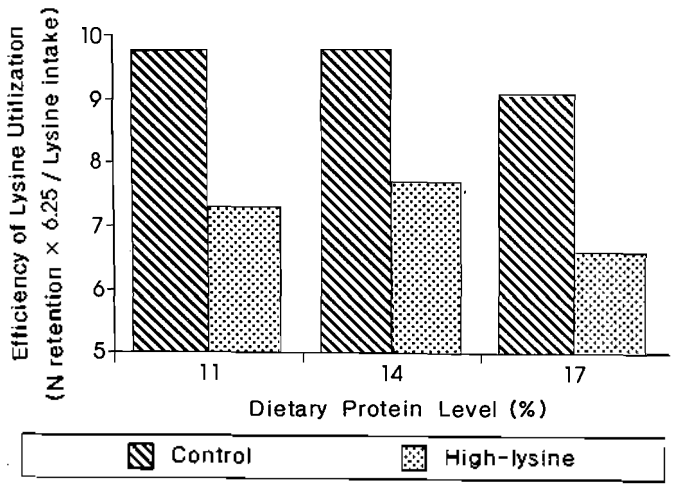

Figure 2. Efficiency of lysine utilization for nitrogen retention in growing pigs weighing 69 kg. The efficiency was estimated by (N retention × 6.25)/lysine intake. The means of lysine efficiency between pigs fed control diets containing 5.2 g lysine/100 g CP and those fed high-lysine diets containing 6.7 g lysine/100 g CP were different ($p < 0.01$). Columns present treatment means (SEM = 0.6, n = 5).

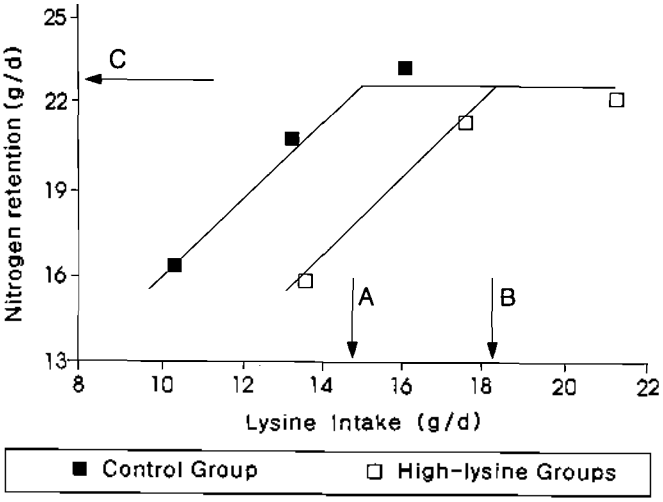

Figure 3. Relationship of lysine intake and nitrogen retention in growing pigs weighing 69 kg. When pigs were fed control diets containing 5.2 g of lysine/100 g CP, N retention of ~23 g/d (indicated by the arrow 'C') was achieved with the lysine intake of 14.5 g/d (indicated by arrow 'A'). When pigs were fed diets containing high-lysine (6.7 g/100 g CP), lysine intake required for the nitrogen retention 'C' shifted to right ('B', ~18.5 g/d). Symbols represent treatment means (SEM = 1.7, n = 5). Means of nitrogen retention were significantly affected by level of protein and lysine ($p < 0.01$).

DISCUSSION

Lysine intake for pigs fed controldiets was 23% less than in pigs fed high-lysine diets. Since the most limiting indispensable amino acids (e.g. methionine + cystine, threonine, tryptophan, valine and isoleucine) were in a constant ratio to lysine for all diets, 23% less lysine intake in pigs fed control diets as compared to lysine intake in pigs fed high lysine diets resulted in correspondingly less intake of other indispensable amino acids at each given protein level. The present study showed that the influence of relative lysine content of

ideal protein on nitrogen retention and efficiency of nitrogen use for N retention were almost identical for two lysine concentrations (5.2 and 6.7 g/100 g CP). Our results disagree with those of Wang and Fuller (1989) and ARC (1981).

The ARC (1981) suggested that the optimum content of lysine was 7.0% of dietary protein, based largely on amino acid composition in pig carcass protein. Wang and Fuller (1989) conducted a N balance study with four different concentrations of lysine (5.0, 6.0, 7.0 and 8.0 g/100 g CP) to determine the optimum concentration of lysine in CP (or indispensable : dispensable amino acids) in growing pigs weighing about 40 kg. Using the broken line method, they concluded that the concentration, 6.5 g of lysine per 100 g ideal protein is optimum for maximum nitrogen retention in growing pigs. They also determined in this experiment that the 6.5% lysine in the ideal protein with the amino acid pattern suggested by Wang and Fuller (1989) was superior to 7.0% lysine in the ideal protein having the amino acid pattern proposed by the ARC (1981).

The crude protein content of the semi-purified diets (nitrogen content × 6.25) used by Wang and Fuller (1989) was about 9% even though the requirement of protein for 20 to 50 kg pigs is over 15% (ARC, 1981 ; NRC, 1988). In addition, nitrogen intake was severely restricted for all pigs used in Wang and Fuller's research. Based on average body weight of pigs used (~40 kg), only 130 g of protein was fed daily to pigs. It was considerably less than the protein requirements of 230 g/d (ARC, 1981) and 280 g/d (NRC, 1988) of pigs of this size.

In Wang and Fuller's study (1989), dietary protein was provided by casein supplemented with synthetic amino acids. For a supply of dispensable amino acids, alanine, aspartic acid and glutamate were used. The nitrogen contents of alanine, aspartic acid and glutamate are 15.72%, 10.52% and 9.52% respectively. The factors for converting nitrogen to amino acid for these three amino acids are 6.4, 9.5 and 10.5, respectively.

The factor for alanine is close to the standard factor (6.25) for converting nitrogen in a complete diet to amino acids, but the factors for two other amino acids (aspartate and glutamate) are much higher than the standard factor. Therefore, the lysine content of Wang and Fuller's diets expressed as a percent of crude protein (16 g N × 6.25) would be different from the content expressed as a percent of total amino acids on the basis of weight.

Chung and Baker (1992) experimentally validated the ideal protein amino acid pattern for 10 kg pigs using a chemically defined diet. Glutamate was the sole source of dispensable amino acids. The lysine content of Chung and Baker's diet was 8.1 g per 16 g N (or 100 g CP), but it was approximately 6.0 g per 100 g amino acids when expressed on the basis of weight. Therefore, the content of lysine per 100 g CP (16 g N × 6.25) in purified or semi-purified diets can not be directly applied to practical diets based on cereals and oil seed meals since excesses can not be adequately controlled.

Since the rate of nitrogen retention for pigs fed high-lysine diets did not improve with an increased intake of lysine and other indispensable amino acids, the efficiency of lysine utilization for nitrogen retention (nitrogen retention/lysine intake) in high-lysine groups was lower ($p < 0.01$) than that in control groups (figure 2). The rate of lysine accretion in whole body was not measured in this nitrogen balance study, so we could not estimate the efficiency of lysine utilization for lysine deposition.

In this nitrogen balance study, the rate of nitrogen retention was maximized at 0.98 g/kg $BW^{0.75}$ per day (or 23.4 g/d). This rate of nitrogen retention for female pigs (average 70 kg BW) is superior to the estimate of Carr et al. (1977) which was 0.83 g/kg $BW^{0.75}$ per day, but similar to the recent assessment (0.93 g/kg $BW^{0.75}$ per day) of Hansen and Lewis (1993) for pigs having the same body size. The nitrogen retention seemed to reach almost plateau in pigs fed high-lysine diets containing 14% and 17% protein. However, there were not enough dietary protein levels to clearly establish this. The efficiency of absorbed nitrogen use for nitrogen retention (ABV) rapidly declined as protein content increased from 14% to 17% ($p < 0.01$). There was also a small increase in nitrogen retention between 14% CP and 17% CP of high lysine diet. This implies that the rate of protein accretion was at or near plateau. The maximum protein accretion was estimated to be 140 to 145 g/d (N × 6.25, table 3) when pigs were fed 17% CP diet (protein intake = 330 g).

As the efficiency of lysine utilization for N retention (nitrogen retention/lysine intake) declined from 1.53 (averaged from all 3 levels of protein, SEM = 0.3, n = 15) for control groups to 1.15 (SEM = 0.3, n = 15) for high-lysine groups (table 3), the apparent requirement of lysine for N retention considerably increased. Pigs fed high-lysine diets utilized ingested lysine for N retention less efficiently than pigs fed control diets (figure 3), so the lysine required to achieve the nitrogen retention shifted from ~14.5 g/d to ~18.5 g/d. Results obtained from our study show that the concentration of 5.2 g lysine per 100 g CP is enough to support maximum nitrogen retention in growing pigs fed practical diets. The prior estimate of 6.5 g lysine per 100 g CP is misleading and would result in a significant economic cost.

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